CLAIMS

1. An expression silencing system comprising

a) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or to a functional equivalent or fragment thereof which sequence carries an NLS sequence, and further comprising at least one promoter and at least one terminator sequence operably linked to said T7-pol; and

b) a second DNA construct comprising a T7 promoter sequence (pT7) or a functional fragment thereof, at least one targeting sequence downstream to said pT7 and at least one 3` non-translated terminator sequence operably linked to said targeting sequence;

which system is capable, upon introduction thereof into a cell, of rendering the expression at the RNA level of a target sequence in said cell, in a tissue or organ regenerated from said cell or in a progeny thereof, substantially silenced, by causing the substantial disappearance of the RNA or RNA transcript carrying said sequence or a functional part thereof.

2. A protein expression silencing system comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, which construct further comprises at least one promoter and at least one terminator sequence operably linked to said T7-pol, a T7 promoter (pT7) or a functional equivalent or fragment thereof, at least one targeting sequence downstream to said the pT7, and at least one additional terminator sequence operably linked to said targeting sequence, said system being capable, upon introduction thereof into a cell, of rendering the expression at the RNA level of a target sequence in said cell, in a tissue or organ regenerated from said cell or in a progeny thereof, substantially silenced, by causing the substantial disappearance of the RNA or RNA transcript carrying said sequence.

3. The expression silencing system as claimed in claim 1 or claim 2, wherein said cell, in which the expression at the RNA level is substantially silenced is an eukaryotic

cell or a prokaryotic cell selected from a plant cell, a mammalian cell, a bacterium, a yeast, their pathogens, or any suitable tissue culture cell.

4. The expression-silencing system as claimed in claim 1 or claim 2, wherein said regenerated organ is a flowering differentiated plant regenerated from said cell.

5. The expression silencing system as claimed in claim 1 or claim 2, wherein said at least one targeting sequence substantially identical or homologous to at least part of said target sequence.

6. The expression silencing system as claimed in claim 5, wherein said target sequence corresponds to:-

a) a gene encoding a protein or a peptide product, the silencing of which is desired;

b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence;

c) a nucleic acid sequence which corresponds to (a) or to (b) or to a fragment thereof, within the scope of degeneracy of the genetic code; or

d) a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.

7. The expression silencing system as claimed in claim 6, wherein said gene encodes a plant protein or peptide product or a protein or peptide product of a plant pathogen.

8. The expression silencing system as claimed in claim 7, wherein said protein or peptide product of a plant pathogen is plant virus, a bacterium or a fungus capable of infecting said plant.

9. The expression silencing system as claimed in claim 7, wherein said gene encodes the GUS protein.

10. The expression silencing system as claimed in claim 6, wherein said gene encodes a human protein or peptide product or a protein or peptide product of a human pathogen.

11. The expression silencing system as claimed in claim 6, wherein said non-coding sequence is a regulatory element sequence which, under normal conditions, promotes the expression of a coding sequence.

12. The expression silencing system as claimed in claim 11, wherein said target sequence is the TMV non-coding sequence Ω.

13. The expression silencing system as claimed in claim 5, optionally further comprising additional regulatory elements.

14. The expression silencing system as claimed in claim 5, wherein said NLS sequence is the SV-40 NLS sequence.

15. The expression silencing system as claimed in claim 5, wherein said promoter sequence is the plant promoter p35S.

16. The expression silencing system as claimed in claim 1 or claim 2, wherein any or both of said terminators is the NOS terminator or a functional equivalent or fragment thereof, the β-1,3-gluconase terminator or any other suitable terminator capable of terminating the transcription of a nucleic acid sequence and of the addition of polyadenylated ribonucleotides to the 3` end of the primary transcript of said nucleic acid.

17. The expression silencing system as claimed in claim 1 or claim 2, wherein said pT7 corresponds to the promoter sequence of the bacteriophage T7 or functional analogues thereof, which promoter is capable of initiating transcription of said at least one targeting sequence downstream thereto.

18. The expression silencing system as claimed in claim 1 or claim 2, comprising the T7 terminator and the NOS terminator operably linked to said targeting sequence.

19. The expression silencing system as claimed in claim 1, wherein said first and second DNA constructs are substantially as shown in Figures 1A and 1B, respectively.

20. A process for the transformation of a plant with a gene-silencing system which process comprises:-

a) transf rming plant cells with:-

i) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof, at least one plant promoter and at least one plant terminator sequence operably linked to said T7-pol;

and with

ii) a second DNA construct comprising a T7 promoter sequence or a functional fragment thereof, a targeting sequence downstream to said T7 promoter, and at least one 3` non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;

b) selecting the plant cells transformed with at least one DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant; and

c) hybridizing a plant transformed with said first DNA construct with a plant transformed with said second DNA construct, which first plant and second plant are obtained in (b), said hybridization thus providing a double-transformed plant in which the expression of a target sequence is substantially suppressed.

21. A process for the transformation of plant with a gene-silencing system, which process comprises:-

a) transforming plant cells with a DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising at least one plant promoter sequence and at least one plant terminator sequence operably linked to said polymerase gene, a T7 promoter sequence (pT7) or a functional fragment thereof, a targeting sequence downstream to said pT7, and at least one additional terminator sequence operably linked to said targeting sequence, which DNA construct is capable, upon transformation thereof into a plant, of rendering the expression of a target sequence in said plant or in its progeny, substantially silenced; and

b) selecting plant cells transformed with said DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant.

22. The process as claimed in claim 20 or claim 21, wherein said targeting sequence substantially corresponds to said target sequence or to a fragment thereof.

23. The process as claimed in claim 22, wherein said target sequence corresponds to:-

a) a gene encoding a protein or a peptide product, the silencing of which is desired;

b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence;

c) a nucleic acid sequence which corresponds to (a) or to (b) or to a fragment thereof, within the scope of degeneracy of the genetic code; or

d) a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.

24. A method for producing a transgenic plant carrying a substantially silent target sequence, by hybridizing a plant carrying and expressing said target sequence with a transformed plant obtained by the process of claim 22.

25. A method for producing a transgenic plant carrying a substantially silent target sequence, by grafting a plant, or parts thereof, carrying and expressing said silent target sequence on a transformed plant obtained by the process of claim 22.

26. A transgenic plant or its progeny obtained by the method of claim 24 or claim 25, in which the expression of said target sequence is substantially suppressed.

27. A method of silencing the expression of a target sequence within the genome of a plant or within the genome of a plant infecting pathogen present in said cell prior to the following manipulation, which method comprises the steps of:

a) providing a first plant capable of regenerating;

b) hybridizing said first plant with a second plant double transformed with:-

i) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct

further comprising at least one plant promoter and at least one plant terminator sequence operably lined to said sequence;

and with

ii) a second DNA construct comprising a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7 and a 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising additional regulatory elements operably linked to said targeting sequence; and

c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is substantially silenced.

28. A method of silencing the expression of a target sequence within the genome of a plant or within the genome of a plant infecting pathogen present in said cell prior to the following manipulation, which method comprises the steps of:-

a) providing a first plant comprising said target sequence, said plant being capable of regenerating;

b) hybridizing said first plant with a second plant transformed with a DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising a plant promoter and a plant terminator sequence operably linked to said T7-pol, a T7 promoter (pT7) or a functional fragment thereof, a targeting sequence downstream to said pT7, and at least one additional promoter sequence operably linked to said targeting sequence; and

c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is substantially silenced.

29. A method as claimed in claim 27 or claim 28, wherein said targeting sequence substantially corresponds said target sequence or to a fragment thereof.

30. The method as claimed in claim 28, wherein said target sequence corresponds to:-

a) a gene encoding a protein or a peptide product, the silencing f which is desired;

b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence;

c) a nucleic acid sequence which corresponds to (a) or to (b) or to a fragment thereof, within the scope of degeneracy of the genetic code; or

d) a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.

31. A method of identifying a nucleic acid of interest within a plant's genome wherein said nucleic acid of interest encodes a pre-defined plant phenotype, which process comprises the steps of:-

a) providing a first plant comprising within its genome said nucleic acid of interest;

b) transforming said first plant with a second plant transformed with:-

i) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising at least one plant promoter and at least one plant terminator sequence operably lined to said sequence;

and with

ii) a second DNA construct comprising a T7 promoter sequence, a random nucleic acid sequence downstream to said T7 promoter, and a 3` non-translated terminator sequence operably linked to said random nucleic acid sequence, said construct optionally further comprising additional regulatory elements operably linked to said nucleic acid of interest, said transformation thus provides a population of transgenic plants;

c) selecting from the population obtained in step (a) those transformed plants/plant cells in which the pre-defined phenotype is substantially silenced; and

d) employing said random nucleic acid sequence within the genome of transformed plants selected in step (c) as a probe in screening genomic DNA and cDNA libraries of said first plant, thereby identifying the gene comprising said random nucleic acid sequence which gene is responsible for said pre-defined phenotype.

32. A method of identifying a nucleic acid of interest within a plant's genome wherein said nucleic acid of interest encodes a pre-defined plant phenotype, which process comprises the steps of:-

a) providing a first plant comprising within its genome said nucleic acid of interest;

b) transforming said first plant with a second plant transformed with a DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising at least one plant promoter sequence and at least one plant terminator sequence operably linked to said T7-pol, said DNA construct further comprising a T7 promoter sequence or a functional fragment thereof, a random nucleic acid sequence downstream to said T7 promoter, and a 3' non-translated terminator sequence operably linked to said random nucleic acid sequence;

c) selecting from the plants obtained in step (b) transformed plants in which the pre-defined phenotype is substantially silenced; and

d) employing said random nucleic acid sequence within the genome of the transformed plants selected in step (c) as a probe in screening genomic DNA or cDNA libraries of said first plant, thereby identifying the gene comprising said random nucleic acid sequence, which gene is responsible for said pre-defined phenotype.